Phytochemistry, Vol 28, No 10, pp 2827–2831, 1989
Printed in Great Britain

0031-9422/89 $3.00+0.00

N-METHOXYANHYDROVOBASINEDIOL FROM *GELSEMIUM ELEGANS*

LONG-ZE LIN,* GEOFFREY A. CORDELL, CHAO-ZHOU NI†‡ and JON CLARDY‡

Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, U.S.A., ‡Department of Chemistry, Baker Laboratory, Cornell University, Ithaca, NY 14853-1301, U.S.A.

(*Received* 18 *November* 1988)

Key Word Index—*Gelsemium elegans*, Loganiaceae, indole alkaloid; *N*-methoxyanhydrovobasinediol; ^{1}H and ^{13}C NMR spectra, X-ray diffraction data.

Abstract—An extract of the whole plant of *Gelsemium elegans* has afforded a new akuammiline-related alkaloid *N*-methoxyanhydrovobasinediol the structure and stereochemistry of which were deduced by spectral methods and confirmed by X-ray diffraction analysis

INTRODUCTION

In previous investigations of *Gelsemium elegans* (Gardn. and Champ.) Benth., we have reported the isolation of several new indole alkaloids [1, 2]. In this report, we wish to present the isolation and structure determination of a new akuammiline-related alkaloid *N*-methoxyanhydrovobasinediol (*N*-methoxytaberpsychine, **1**).

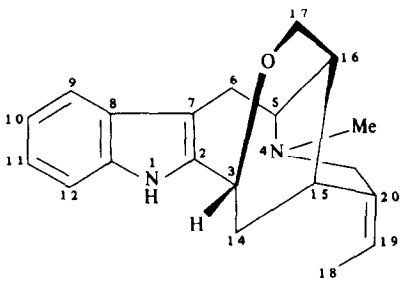
RESULTS AND DISCUSSION

Alkaloid **1** was obtained as white needles. Its UV absorptions at 224 and 282 nm indicated it possessing a 2,7-dehydroindole nucleus, and the ^{1}H NMR spectrum showed signals for an olefinic proton, a methoxy group, a *N*-methyl, four aromatic protons and 12 aliphatic protons, which were similar to those reported for anhydrovobasinediol (**2**), except that **1** has a signal for an *N*-methoxy group at δ4.08 [3–5]. The mass spectrum of the alkaloid displayed a molecular ion at *m/z* 338 ($C_{21}H_{26}N_2O_2$), 30 mass units more than that of anhydrovobasinediol (**2**), supporting the notion that **1** might be the *N*-methoxy derivative of **2**.

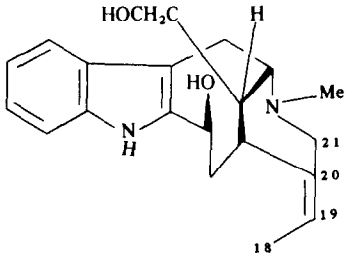
The ^{13}C NMR and APT spectra of **1** indicated the presence of five quaternary, nine methine, four methylene and three methyl carbon atoms. Unambiguous assignments were obtained using APT, CSCM 1D [6] and selective INEPT [7] pulse programming sequences. In the CSCM 1D experiment, an upfield or downfield carbon satellite of a proton signal is irradiated and magnetization is transferred to the attached carbon. In the *J*-modulated selective INEPT pulse sequence experiment, a particular proton is irradiated with a soft pulse resulting in a magnetization transfer and a selective enhancement of the signals of carbon atoms three bonds away from the irradiated proton. CSCM 1D irradiation of the ^{13}C satellites of H-9 (δ7.63), H-10 (δ7.15), H-11 (δ7.25), H-12 (δ7.40), H-19 (δ5.53), H-3 (δ5.41), OMe (δ4.08), H-5 (δ3.10), H-15 (δ2.85), NCH_3 (δ2.58), H-16 (δ2.50) and H-18 (δ1.60) of **1** resulted in magnetization transfer to their corresponding carbon atoms appearing at δ118.21 (C-9), 119.45 (C-10) 122.51 (C-11), 108.15 (C-12), 119.80 (C-19), 63.66 (C-3), 65.48 (OMe), 60.29 (C-5), 33.27 (C-15), 42.88 (NMe), 37.27 (C-16) and 12.68 (C-18), respectively. CSCM 1D experiments also led to the assignment of the following methylene groups: H-14 and C-14 at δ2.05, 2.48 and 29.82; H-17 and C-17 at δ3.46, 3.86 and 61.63; H-6 and C-6 at δ3.15, 3.40 and 17.98; H-21

*On leave of absence from Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, People's Republic of China

†On leave of absence from Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai, People's Republic of China



2a published structure of anhydrovobasinediol



8a published structure of vobasinediol

and C-21 at δ3 38, 3 90 and 45 87 Selective INEPT irradiation of H-14β (δ2 05) enhanced the signals at δ33 27 (C-15), 135 90 and 130.24, two of which could be assigned to C-20 and C-2 These quaternary carbons could be distinguished when H-18 (δ1.60) was irradiated resulting in the selective enhancement of the signal for C-20 (δ135 90) Polarization transfer from H-9 (δ7.63) enhanced the C-13 (δ131 63), C-11 (δ122 51) and C-7 (δ107.14) signals, and irradiation of H-10 (δ7 15) resulted in enhancement of the C-8 (δ122 95) and C-12 (δ108 15) signals Irradiation of H-11 (δ7 25) enhanced the C-13 (δ131 63) and C-9 (δ118 21) signals, and irradiation of NMe (δ2.58) enhanced those of C-5 (δ60 29) and C-21 (δ45 87) The complete assignments of the ^{13}C NMR spectra of **1** are shown in Table 1

Recently, it was established by difference NOE experiments that the 19,20-double bond of koumidine (**3**) possessed the *Z*-configuration [8]. The observation of a strong NOE (6.5%) between H-18 and H-21 in *N*-methoxyanhydrovobasinediol (**1**) shows that it too has the *Z*-configuration at C-19,20

The structure and relative stereochemistry were confirmed by single crystal X-ray diffraction analysis Figure 1 is a perspective drawing of the final X-ray model with the hydrogens The absolute configuration shown was set by biogenetic considerations, C-15 (*R*), outlined later in the paper. Table 2 contains the fractional coordinates of

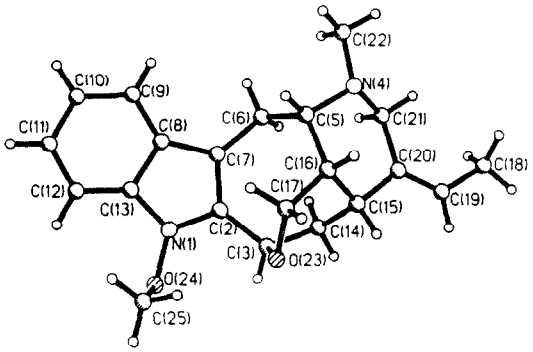


Fig 1

the heavy atoms, and Tables 3 and 4 list the bond lengths and bond angles, respectively Anisotropic displacement coefficients, H-atom coordinates and isotropic displacement coefficients and torsional angles are available from the authors [9] The absolute configurations shown in the X-ray illustration and in **1** are identical, but the real conformation shown in the computer drawing differs substantially from the conventional mode of drawing shown in **1** The six-membered piperidine ring (N-4, C-5, C-16, C-15, C-20, C-21) is in a chair conformation while

Table 1 ^{1}H and ^{13}C NMR data for compound **1**

C	^{1}H	^{13}C
2	-	130 24
3	5 41 (*d*, 9 6)	63 66
5	3 10 (*m*)	60 29
6α	3 15 (*m*)	17 98
6β	3 40 (*m*)	
7	-	107 14
8	-	122 95
9	7 63 (*d*, 7 8)	118 21
10	7 15 (*t*, 7 8)	119 45
11	7 25 (*t*, 7 8)	122 51
12	7 40 (*d*, 7 8)	108 15
13	—	131 63
14α	2 05 (*m*)	29 82
14β	2 48 (*m*)	
15	2 85 (*m*)	33 27
16	2 50 (*m*)	37 27
17α	3 86 (*dd*, 10 5, 9 8)	61 63
17β	3 46 (*dd*, 10 5, 9 8)	
18	1 60 (*d*, 6 3)	12 68
19	5 53 (*q*, 6 3)	119.80
20	—	135 90
21α	3 90 (*m*)	45 87
21β	3 38 (*m*)	
N_b-Me	2 58 (*s*)	42 88
N_a-OMe	4 08 (*s*)	65 48

*Recorded in $CDCl_3$, chemical shift values are reported as δ values (ppm) from internal TMS at 300 MHz, signal multiplicity and coupling constants (Hz) are shown in parentheses Carbon chemical shifts are reported as δ values (ppm) at 90 8 MHz

Table 2 Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement parameters ($A^2 \times 10^3$) for **1**

	x	*y*	*z*	U(eq)*
N(1)	7583(5)	8765	5890(4)	45(2)
C(2)	6482(6)	8762(11)	4838(6)	42(3)
C(3)	5503(6)	7344(12)	4576(6)	45(3)
N(4)	5002(5)	9821(11)	808(4)	46(2)
C(5)	5002(6)	9228(11)	1847(6)	47(3)
C(6)	5547(6)	10636(11)	2884(5)	42(3)
C(7)	6551(6)	10087(12)	4080(5)	42(3)
C(8)	7779(5)	10895(12)	4754(5)	38(3)
C(9)	8404(6)	12298(12)	4500(6)	49(3)
C(10)	9646(6)	12704(13)	5374(6)	55(3)
C(11)	10244(7)	11797(13)	6464(7)	63(4)
C(12)	9638(6)	10443(13)	6736(6)	54(3)
C(13)	8428(6)	10033(11)	5908(6)	46(3)
C(14)	4122(6)	7897(12)	3724(5)	45(3)
C(15)	3690(5)	7178(12)	2450(5)	40(3)
C(16)	4742(6)	7278(12)	2115(6)	48(3)
C(17)	5892(6)	6168(12)	3029(5)	49(3)
C(18)	117(8)	8266(16)	306(5)	83(4)
C(19)	1395(6)	7449(13)	1189(6)	56(3)
C(20)	2510(6)	8116(12)	1504(6)	43(3)
C(21)	2726(6)	9904(12)	1045(6)	55(3)
C(22)	3934(7)	11556(12)	291(6)	68(4)
O(23)	5811(4)	5775(10)	4091(4)	55(5)
O(24)	7928(4)	7484(10)	6795(4)	59(2)
C(25)	8564(7)	5988(14)	6579(6)	69(4)
C(26)	7825(8)	1326(16)	1377(8)	90(5)
O(27)	7085(6)	2776(13)	1339(5)	99(3)

*Equivalent isotropic U defined as one-third of the trace of the orthogonalized U_{ij} tensor

Table 3 Bond lengths (Å) for compound **1**

	Bond lengths (Å)		Bond lengths (Å)
N(1)–C(1)	1 356(7)	N(1)–C(13)	1.381(9)
N(1)–O(24)	1 391(7)	C(2)–C(3)	1.498(11)
C(2)–C(7)	1.404(11)	C(3)–C(14)	1.544(8)
C(3)–O(23)	1.448(11)	N(4)–C(5)	1.467(7)
N(4)–C(21)	1 485(11)	N(4)–C(22)	1.484(12)
C(5)–C(6)	1 557(10)	C(5)–C(16)	1 560(12)
C(6)–C(7)	1.472(8)	C(7)–C(8)	1 440(9)
C(8)–C(9)	1 411(12)	C(8)–C(13)	1.433(9)
C(9)–C(10)	1.404(8)	C(10)–C(11)	1 385(11)
C(11)–C(12)	1.380(13)	C(12)–C(13)	1 362(8)
C(14)–C(15)	1 528(9)	C(15)–C(16)	1.511(11)
C(15)–C(20)	1.522(8)	C(16)–C(17)	1.553(9)
C(17)–O(23)	1 415(10)	C(18)–C(19)	1.531(10)
C(19)–C(20)	1 302(10)	C(20)–C(21)	1.525(13)
O(24)–C(25)	1 451(12)	C(26)–O(27)	1 388(15)

Table 4 Bond angles (°) for compound **1**

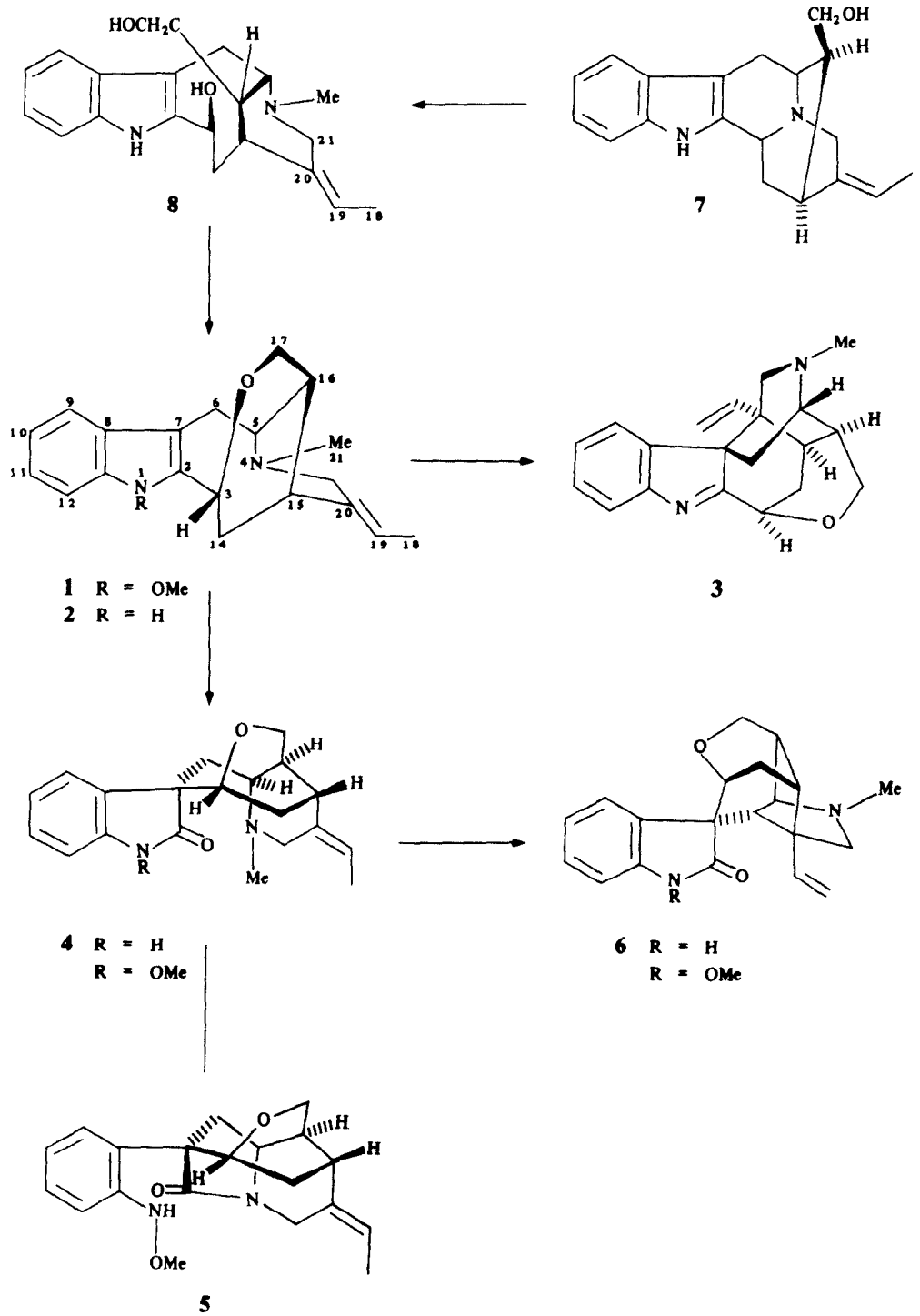
	Bond angles (°)		Bond angles (°)
C(2)–N(1)–C(13)	112 8(5)	C(2)–N(1)–O(24)	124 8(5)
C(13)–N(1)–O(24)	121 8(4)	N(1)–C(2)–C(3)	121 2(6)
N(1)–C(2)–C(7)	108.5(7)	C(3)–C(2)–O(23)	129.8(5)
C(2)–C(3)–C(14)	115 9(7)	C(2)–C(3)–O(23)	109 5(6)
C(14)–C(3)–O(23)	109.3(5)	C(5)–N(4)–C(21)	114 1(6)
C(5)–N(4)–C(22)	113 9(6)	C(21)–N(4)–C(22)	109 0(7)
N(4)–C(5)–C(6)	112.7(6)	N(4)–C(5)–C(16)	105 6(6)
C(6)–C(5)–C(16)	119.8(6)	C(5)–C(6)–C(7)	118 9(7)
C(2)–C(7)–C(6)	127.4(6)	C(2)–C(7)–C(9)	105.6(5)
C(6)–C(7)–C8)	126 9(7)	C(7)–C(8)–C(9)	133 2(6)
C(7)–C(8)–C(13)	108.8(7)	C(9)–C(8)–C(13)	118 0(5)
C(8)–C(9)–C(10)	117.7(6)	C(9)–C(10)–C(11)	122.0(8)
C(10)–C(11)–C(12)	121.0(6)	C(11)–(12)–C(13)	118 2(7)
N(1)–C(13)–C(8)	104.4(5)	N(1)–C(13)–C(12)	132 6(7)
C(8)–C(13)–C(12)	122.9(8)	C(3)–C(14)–C(15)	110 6(6)
C(14)–C(15)–C(16)	111.2(5)	C(14)–C(15)–C(20)	112.6(7)
C(16)–C(15)–C(20)	110.5(6)	C(5)–C(16)–C(15)	112.8(7)
C(5)–C(16)–C(17)	117 4(6)	C(15)–C(16)–C(17)	109 6(6)
C(16)–C(17)–O(23)	111 9(7)	C(18)–C(20)–C(21)	126 9(9)
C(15)–C(20)–C(21)	120.2(8)	C(15)–C(20)–C(21)	115.9(6)
C(19)–C(20)–C(21)	123 7(6)	N(4)–C(21)–C(20)	111 5(7)
C(3)–O(23)–C(17)	112 2(7)	N(1)–O(24)–C(25)	110 9(6)

the six-membered oxygen-containing ring (C-3, C-14, C-15, C-16, C-17, O-23) is in an almost ideal boat. The stereochemistry of the C-20, C-19 double bond is *Z*, i.e the C-18 methyl group is on the same side as C-21.

According to biogenetic considerations, *Gelsemium* alkaloids such as koumine (**3**), the humantenines (**4**), gelsemamides (**5**) and gelsemines (**6**) may be formed from koumidine (**7**) via vobasinediol (**8**) through anhydrovobasinediol (**2**) [10]. Therefore vobasinediol (**8**) and anhydrovobasinediol (**2**) should have the same *Z*-configuration of the side chain double bond as koumidine (**7**), the humantenines (**4**) and the gelsemamides (**5**). Because so many *Gelsemium* alkaloids possess the *N*-methoxy group, the first isolation of *N*-methoxyanhydrovobasinediol (**1**) from this genus is of substantial biogenetic interest as it may represent an important early intermediate on a separate pathway to the *N*-methoxy *Gelsemium* alkaloids. As a result of this work, the C-19, C-20 stereochemistry of vobasinediol (**8**) and anhydrovobasinediol (**2**) may need to be revised.

EXPERIMENTAL

Mp: uncorr ^{1}H NMR and homonuclear COSY spectra: $CDCl_3$, using TMS as int standard; ^{13}C NMR: 90.8 MHz; low resolution mass spectrum. 70 eV; CD spectrum: MeOH; X-ray



data were recorded on a Nicolet R3 diffractometer and analysed on a Microvax II c p.u using the SHELXTL series of programs.

Plant material Whole plants of *Gelsemium elegans* were collected in Guangxi Province of China in February, 1987, and voucher specimens are deposited in the herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, People's Republic of China

Extraction and fractionation. The air-dried plant material (20 kg) was percolated with EtOH (200 l) at room temp and the EtOH extract concd *in vacuo* at 50° to afford a thick dark syrup, which was dissolved as far as possible in 1% HCl soln, and the residual solid treated with 1% HCl until a Dragendorff's test was negative After extraction (× 3) with $CHCl_3$, the acidic layer was basified with NH_3 and extracted (× 5) with $CHCl_3$ to give crude alkaloid extract A (112 g)

Isolation of N-methoxyanhydrovobasinediol (1) A portion of the 1% MeOH in $CHCl_3$ eluent (1 00 g) from the CC (Column 3) [1] of the alkaloid extract (A) was subjected to repeated prep

TLC using cyclohexane–EtOAc–diethylamine (8.2:1 as a solvent system. The second band at R_f 0.75 was eluted with Me_2CO to afford white needles of **1** (80 mg), mp. 75°, $[\alpha]_D$ −272.5° (*c* 0.24, MeOH); UV λ_{max} (log ε) 203 (4.58), 224 (4.56) and 282 (3.63) nm; IR ν_{max}^{KBr} 2937, 2913, 2904, 2862, 1088, 1073, 1055, 1041, 739 cm^{-1}, ^1H and ^{13}C NMR, see Table 1, MS *m/z* (rel. int.) 338 $[M]^+$ (8), 308 (83), 307 (22), 293 (20), 279 (10), 198 (12), 168 (12), 159 (11), 158 (11), 156 (12), 154 (21), 152 (12), 144 (11), 143 (11), 136 (11), 134 (10), 130 (24), 128 (12), 122 (100), 121 (58), 120 (31), 108 (11), 107 (15), 77 (29), CD (MeOH) Δε (nm) −14.08 (230), −2.56 (280)

X-Ray analysis of N-methoxyanhydrovobasinediol (**1**). Crystals formed in the monoclinic system with $a = 12.015$ (5), $b = 7.469$ (3), $c = 12.576$ (6) Å and $\beta = 118.32$ (2)°. Systematic extinctions and density considerations were uniquely accommodated with space group $P2_1$ and a unit of composition $C_{21}H_{26}N_2O_2 \cdot CH_3OH$ (370.496) forming the asymmetric unit ($Z = 2$, $D_c = 1.24$ g/cm^{-3}). All unique diffraction maxima with $2\Theta \leq 110°$ were collected using Θ–2Θ scans and CuKα radiation (1.54178 Å). Of the 1365 reflections measured, 1246 (91%) were judged observed $[|F_o| \geq 4\sigma(F_o)]$ and used in subsequent calculations. No correction was made for absorption [μ(CuKα) = 6.21 cm^{-1}]. The structure was solved by direct methods using the SHELXTL implementation and refined by full-matrix least-squares refinements to a final agreement factor of 0.052. The final model had anisotropic non-hydrogen atoms and isotropic hydrogens which rode on the non-hydrogen atoms at standard geometries

Acknowledgements—This work was supported, in part, by grants (CA20164 to GAC and CA24487 to JC) from the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. The authors thank the Research Resources Center of the University of Illinois at Chicago for the provision of NMR and mass spectroscopic facilities. The work at Cornell University was also partially supported by NIH grant RR04150

REFERENCES

1 Lin, L.-Z., Cordell, G. A., Ni, C.-Z. and Clardy, J. (1989) *J. Nat. Prod.* (in press)
2 Lin, L.-Z., Cordell, G. A., Ni, C.-Z. and Clardy, J. (1989) *J. Org. Chem.* (*in press*)
3. Burnell, R. H. and Medina, J. D. (1971) *Can. J. Chem.* **49**, 308
4. van Beek, T. A., Kuijlaars, F. L. C., Thomassen, P. H. A. M., Verpoorte, R. and Baerheim Svendsen, A. (1984) *Phytochemistry* **23**, 1771
5. van Beek, T. A., Verpoorte, R. and Baerheim Svendsen, A. (1985) *J. Nat. Prod.* **48**, 400
6 Sarkar, S. K. and Bax, A. (1985) *J. Magn. Res.* **62**, 109
7 Bax, A. (1984) *J. Magn. Res.* **57**, 314.
8 Yeh, S. and Cordell, G. A. (1987) *Phytochemistry* **26**, 5875
9 The main library of programs used for data collection and analysis were the Nicolet/SHELXTL library distributed by Nicolet Instrument Division, Madison, Wisconsin
10 Liu, Z.-J. and Lu, R.-R. (1988) *Alkaloids*, **33**, 83.